Short communication

Marked differences between MARC-145 cells and swine alveolar macrophages in IFNβ-induced activation of antiviral state against PRRSV

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ARTICLE INFO

ABSTRACT

The activation of antiviral activity induced by recombinant swine interferon beta (rswIFNβ) against PRRSV was comparatively examined in MARC-145 cells and porcine alveolar macrophages (PAMs). A dose–response analysis showed, in MARC-145 cells, that isolate Mo25544 was highly sensitive to rswIFNβ while a vaccine strain and isolate PDV130-9301 were resistant to different extents. In contrast, all three viruses were equally sensitive to rswIFNβ in PAMs even at the lowest dose of IFN utilized in the bioassays. To analyze potential differences in mechanisms of antiviral activation between these cells, treatment with 2-aminopurine (2-AP), an inhibitor of double-stranded RNA-dependent protein kinase (PKR), was performed in rswIFNβ-treated cells. Addition of 2-AP to rswIFNβ-primed MARC-145 cells restored replication of the Mo25544 isolate, and to some extent that of vaccine virus and PDV130-9301. In contrast, virus replication could not be rescued for any of the three viruses with 2-AP in rswIFNβ-treated PAMs. The differences in sensitivity of PRRSV to rswIFNβ as well as the effects of 2-AP strongly suggest that MARC-145 cells and PAMs utilize different rswIFNβ-associated antiviral pathways. Therefore, studies to understand virus-host cell interactions performed in MARC-145 cells require additional scrutiny when utilized as a host cell model for immunologic responses to PRRSV.

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1. Introduction

Porcine reproductive respiratory syndrome (PRRS) is one of the most important viral diseases that negatively affect the swine industry worldwide (Keffaber, 1989; Murtaugh et al., 2002; Mengeling et al., 2003). The causative agent, porcine reproductive respiratory syndrome virus (PRRSV), is a member of the Arteriviridae family in the order Nidovirales. The virus genome is a positive sense single-stranded RNA, and the virion's nucleocapsid is covered by a lipid bilayer containing several viral proteins.

The virus–host relationship is unique, and can be characterized by a deficient innate immune response including poor induction of type I interferon (IFNα/β) (Albina et al., 1998), sub-optimal humoral and cellular immunity, and persistence of the virus for long periods post-infection (Horter et al., 2002; Meier et al., 2003). Effective control of PRRS remains elusive and underscores the need for in-depth studies to gain an insight into the mechanisms leading to such an inefficient host response.

The innate immune response, particularly regarding the modulation and activation of IFNα/β, is relevant to the understanding of host–virus interactions and the resulting immune response (Albina et al., 1998; Alexopoulou et al., 2001; Samuel, 2001). Mounting evidence shows that infection with PRRSV results in poor induction of IFNα critically affecting the ensuing adaptive responses with

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delayed IFNγ and neutralizing antibody production, leading to persistent infection (Albina et al., 1998; Buddaert et al., 1998; Bautista and Molitor, 1999; Aasted et al., 2002; Royaee et al., 2004; Xiao et al., 2004). *In vitro* infection of porcine alveolar macrophages (PAMs) (Genini et al., 2008), monocyte-derived dendritic cells (DCs) or lung DCs (Loving et al., 2006a) resulted in transcription of IFNβ. However, transcription of interferon-stimulated genes, including IFNα, was not upregulated which led to the assumption that IFNβ may be transcribed but not translated (Loving et al., 2006a) thus preventing downstream signaling events. However, the assumed lack of translation of IFNβ remains to be confirmed.

Previously, we have demonstrated the antiviral effects of recombinant IFNβ (rswIFNβ) against PRRSV (Overend et al., 2007) which is consistent with similar findings using IFNα (Lee et al., 2004). As shown with IFNα (Lee and Kleiboeker, 2005), our study also suggested differences in sensitivity to rswIFNβ among PRRSV isolates. MARC-145, an extensively used cell line in the studies of PRRSV-host cell interactions including type I IFN and type II IFN antiviral mechanisms, are of simian origin, and are likely not an appropriate model for such studies. Consequently, the present study was undertaken to define differences between MARC-145 cells and PAMs regarding type I IFN-induced activation. Differences between cell types in sensitivity to IFN type I and effects of 2-aminopurine (2-AP), an inhibitor of double-stranded RNA-dependent protein kinase (PKR), on viral replication are discussed.

Field isolate Mo25544 kindly provided by Dr. Steve Kleiboeker (University of Missouri), PDV130-9301 (NVSL, Ames, IO) and a vaccine virus (RespPRRS Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO) were examined in the study. MARC-145 cells, passage 49–55, were used for bioassays, propagation and titration of viruses. The cells were cultured and maintained in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.35% glucose, 2 mM L-glutamine, 2.5 μg/ml fungizone, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were kept at 37 °C in a humidified 5% CO_2 incubator.

Alveolar macrophages (PAMs) were collected by bronchioalveolar lavage from lungs of PRRSV-negative, 4–6-week-old pigs as previously described (Overend et al., 2007). The PAMs were resuspended in RPMI-1640 with 10% FBS, 2 mM l-glutamine, 1 μg/ml fungizone, 100 U/ml penicillin and 100 μg/ml streptomycin. Before use, the PAM were confirmed to be PRRSV-free by real time RT-PCR. For testing, the cells were plated at a density of 1×10^6 cells/well in 24-well culture plates. The PAM cultures were incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator and washed once with RPMI-1640 complete media before use.

A recombinant replication-defective adenovirus expressing swine IFNβ (Ad5swIFNβ) was the source of rswIFNβ for the study (Chinsangaram et al., 2003). The recombinant virus was propagated in HEK-293 cells (ATCC) with DMEM media containing glucose, antibiotics and 5–10% FBS. Forty-eight to seventy-two hours post-infection (hpi), rswIFNβ-containing culture fluids were harvested. The media was centrifuged at $100,000 \times g$ for

2 h. The resulting supernatant was acidified to pH 2.0 for 18–20 h at 4 °C and then neutralized to pH 7.2 and stored at −80 °C until further use. Culture fluids from control Ad5 virus (Ad5Blue)-infected HEK-293 cells were processed identically and used as a negative control.

Bioassays were performed as previously described by Overend et al. (2007). Briefly, MARC-145 cells or PAM were primed with concentrations of either rswIFNβ, ranging from 20 to 1000 U/well, or an equivalent dilution of Ad5Blue control culture supernatant and then incubated for 20 h. Cells were inoculated with PRRSV isolates at 1000 $TCID_{50}$/well. After 2 h of incubation and washing with serum-free media, media with 2% FBS was added to maintain the cells. Samples were harvested 48 hpi for titration in MARC-145 cells. The activity of rswIFNβ (Units/ml) was based on protection of MARC-145 cells (PD_{50}) from infection with a PRRSV isolate previously determined to be sensitive to rswIFNβ (Overend et al., 2007). The dilution giving 50% protection from infection to the cells was assessed a value of I U from which the titer of the stock preparation (U/ml) was calculated. Confluent MARC-145 cells or PAMs seeded in 24-well plates were primed with rswIFNβ diluted in serum-free DMEM and incubated for 20 h. The cells were inoculated with PRRSV isolates at 1000 $TCID_{50}$/well. After 2 h incubation and subsequent washes, media with 2% FBS, with or without 8 mM 2-AP, was added to maintain the cells. At 48 hpi supernatants and cell pellets were harvested and titrated in MARC-145 cells.

The activation of innate antiviral activity induced by rswIFNβ against PRRSV was comparatively examined in MARC-145 cells and PAMs. Marked differences between the cell types were revealed in the bioassays indicating that increased scrutiny is needed when performing assays in the MARC-145 cell model. The results in MARC-145 cells showed consistently that isolate Mo25544 was sensitive to rswIFNβ while the vaccine strain and the PDV130-9301 isolate were resistant, albeit to different extents (Fig. 1A). Though the vaccine virus was shown before to be sensitive to rswIFNβ in MARC-145 cells (Overend et al., 2007), a dose-dependent resistance was revealed in the present study when rswIFNβ was titrated. In contrast, all isolates tested (PDV130-9301, Mo25544, and vaccine virus) were completely sensitive to all the concentrations of rswIFNβ utilized in PAMs (Fig. 1B). Previous work has demonstrated that the antiviral effects on the cells are induced specifically by rswIFNβ as demonstrated by neutralization of antiviral activities by an anti-rswIFNβ monoclonal antibody (Overend et al., 2007). This study also showed that culture supernatants from HEK-293 cells infected with the empty Ad5 vector had no antiviral effect (Overend et al., 2007). Though type I IFNs are associated with an antiviral function against PRRSV (Lee et al., 2004; Loving et al., 2006a; Overend et al., 2007) the sensitivity of the virus to type I IFN is highly variable. The importance of sensitivity or resistance to type I IFN in the pathogenesis of PRRS remains to be determined. Also, the reasons for the observed differences in rswIFNβ-induced antiviral activation between these cells are presently unknown. A possible explanation is that the relative sensitivity of cells to IFN derived from the same species much greater than to heterologous IFN (Gifford, 1963; Moehring and Stinebring, 1970; Veomett
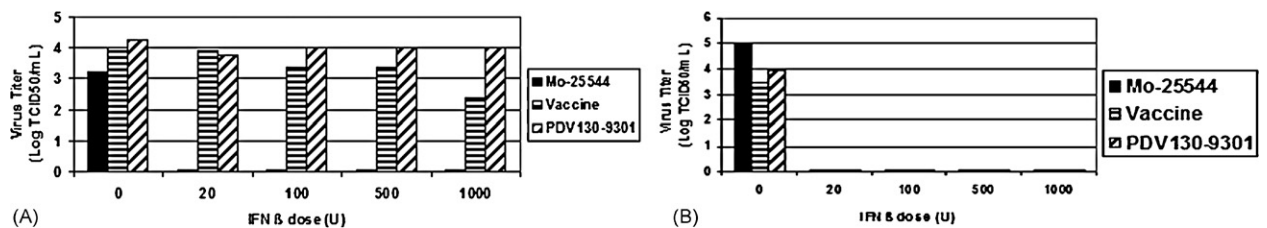
Fig. 1. Dose–dependent sensitivity of various PRRSV isolates to rswIFNβ in MARC-145 (A) cells or PAMs (B). Cells seeded in 24-well culture plates were primed with rswIFNβ at different concentrations (ranging from 20 to 1000 U/well in 100 μL media) and incubated for 20 h. The cells were then inoculated with the indicated isolates (1000 TCID$_{50}$/well) for 2 h. The cells were then rinsed and replenished with media containing 2% FBS. Culture fluids and cells were harvested 48 hpi for titration in MARC-145 cells. Each virus isolate was titrated directly in untreated MARC-145 cells to serve as positive controls and non-infected cells served as negative controls. Titers are in TCID$_{50}$/ml. Zero hour time point control samples (4th wash collected) contained no detectable virus (data not shown).
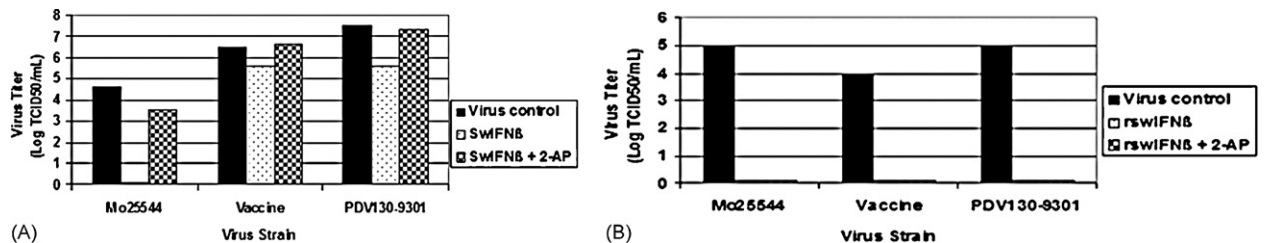

Fig. 2. Effect of 2-AP on PRRSV replication in rswIFNβ-primed MARC-145 cells or PAMs. Confluent MARC-145 cells (A) or PAMs (B) seeded in 24-well plates, were primed with rswIFNβ at 500 U and incubated for 20 h. Cells were challenged with the indicated isolates at 1000 TCID$_{50}$/well for 2 h. After rinsing, media containing 2% FBS, with or without 8 mM 2-AP was added. Culture fluids and cells were collected 48 hpi and titrated on MARC-145 cells. Titers are in TCID$_{50}$/ml. Zero hour time point control samples (4th wash collected) contained no detectable virus (data not shown).

and Veomett, 1979). Further investigation is required to acquire a better understanding of the observed effects on host cells in this system.

A previous study showed that PRRSV replication was inhibited by IFNγ and was partially recovered by addition of 2-AP (Rowland et al., 2001). These observations indicate that PKR is important in the antiviral response of MARC-145 cells against PRRSV. In the current study, the effect of 2-AP on virus replication in rswIFNβ-primed cells was markedly different between these two cells. Treatment of rswIFN β-primed and PRRSV-infected MARC-145 cells with 2-AP restored the replication of the rswIFN β-sensitive isolate (Mo25544), and to some extent those of the vaccine virus and the rswIFNβ-resistant isolate (PDV130-9301) (Fig. 2A). Thus, the results with MARC-145 cells are consistent with PKR being a mechanism of type I IFN-induced cell protection, specifically induced by rswIFNβ. In contrast, an identical treatment of rswIFN β-primed PAMs with 2-AP had no effect on the antiviral state induced resulting in failure to rescue replication of any of the viruses (Fig. 2B). These findings are consistent with a recent report that highlighted differences between poly I:C treated PAMs and peritoneal macrophages (PMs), exhibiting no induction of PKR in the former, and a profound expression in the latter (Loving et al., 2006b). Similarly, Gudmundsdottir and Risatti (2009) reported a lack of PKR expression in PAMs following PRRSV infection. At this point, it is unknown which interferon-stimulated genes are responsible for conferring the observed antiviral state against PRRSV in PAMs. However, while the specific mechanism of swIFNβ-induced protection in PAMs remains uncertain, this study indicates that PKR is not involved in establishing it.

A further understanding of the mechanisms of type I IFN suppression observed in PRRSV-infected animals (Albina et al., 1998; Buddaert et al., 1998), a phenomenon that is becoming increasingly accepted as a contributing mechanism in the virulence and pathogenesis of PRRS, is crucial to the development of effective control measures. Analysis of the differences in sensitivity to rswIFNβ in different cells, observed here, underscores the importance of utilizing appropriate, natural host cells when studying PRRSV. Despite challenges associated with utilizing primary cells versus established cell lines, doing so will provide relevant insights into potential mechanisms of virus/host cell interactions and immune evasion by PRRSV. In this study all the experiments were replicated at least three times utilizing PAMs from different animals with consistent results.

Conflict of interest

On behalf of all co-authors, the corresponding author affirms that there is no conflict of interest with this publication.

Acknowledgements

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